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A comparative study of the effects of acute and chronic gamma irradiation on total soil respiration

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With 2 Figures

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1. Introduction

Gamma radiation has found considerable use in community ecology as a controllable, reproducible stressing agent (WOODWELL and REBUCK 1967). Several studies have been made of the effects of acute radiation from a portable gamma source on above-ground vegetation (MONK 1966; McCORMICK and GOLLEY 1966). Apart from atomic tests, where blast and radiation effects have been inseparable, there have been very few studies of radiation effects on litter and soil organisms in the field (COWLEY 1969; WITKAMP 1961). Both of these studies lacked accurate dosimetry of radiation received in the soil.

Gamma radiation is a useful tool for the study of effects of partial sterilization of the soil, since it alters many physical-chemical properties of litter and soil less than chemical sterilants or heat (ENO and POPENOE 1964). Changes in rates of evolution of CO_2 from the community of small organisms present in litter-soil or total soil respiration, can be measured as an indication of the effects of radiation. From previous laboratory studies, we anticipated that an 800 KR (Kilo-Roentgens) dosage would kill all small arthropods (EDWARDS 1968) and that the 2500 KR dose would kill virtually all organisms if recolonization was prevented (COLEMAN and MACFADYEN 1966; POPENOE and ENO 1962). Thus 800 KR should alter the respiration of litter-soil cores in a different fashion from 2500 KR.

The present study was undertaken to investigate the effects of gamma radiation on the litter-soil community under field and laboratory conditions, comparing the effects of long-term (chronic) doses of radiation in the field with those of short-term (acute) dosages given at a more intense laboratory source.

2. Methods

2.1. Soil respiration measurements

Soil respiration measurements were made by a modified Conway method similar to that of WITKAMP (1963), except that the litter-soil samples were sealed in polyethylene bags (0.05 mm thickness) to prevent recolonization of the samples by organisms. Respiration measurements were not affected by the sealing, as preliminary tests demonstrated that gas molecules could freely diffuse through the bags.

To measure CO_2 evolution, a 125 cc sealed core of litter-soil was placed in a container, a jar of dilute NaOH placed over it, and the container closed. After several hours (usually 22—24), the absorbed CO_2 was precipitated with $BaCl_2$, and the excess NaOH titrated with 0.100 N HCl, using a GILMONT micrometer syringe, accurate to 0.002 m*l*.

2.2. Irradiation methodology

Field irradiation was done with a 9000-curie portable ^{137}Cs source (McCormick and Golley 1966), beamed downward at 15° from the vertical, to intensify the dosage received by the soil. The source was set in a small clearing in a hardwood forest area of the Savannah River Plant, with a litter layer of predominantly Southern red Oak [*Quercus falcata* (Michx.)]. Two portions, 75 m^2 ea., were set aside as irradiated and control (non-irradiated) areas. The radiation area was then marked off at 0, 11, 22 and 45 degrees to each side of the midline of the direction faced by the source. The source was opened on June 14, 1968, and closed briefly only at the beginning and end of dosimetry checks. Lithium fluoride (Toshiba Low Z) glass rod microdosimeters were placed at 1, 2, 4 and 8 m. distances on the grid lines, on the surface and 5 cm. deep for six hours and twenty-four hours to measure doses received per hour and total dosage. The rods were contained in aluminum-alloy capsules to eliminate the low-energy Beta scatter, or "Bremsstrahlungs-Effect". The glass rods were read in a Bausch and Lomb microdosimeter reader, accurate to ± 5%.

Fifteen cores of litter-soil which had received 800 KR were taken six weeks after the source had been opened. Four sets of 15 samples were taken at random from the control area at the same time. All cores were taken at a 0—5 cm. depth. Three groups were taken to a 176,000-curie, ^{60}Co source at the Savannah River Laboratory of the Savannah River Plant. One group received 800 KR, another received 2500 KR, and the third served as a test of the effect of moving, or transportation controls. All samples were then taken to the laboratory for measurement of respiration. Ten cores were analyzed from each group, including the field radiation group which received 800 KR and a control set previously left in a controlled temperature box. The rates of respiration were checked four hours after the acute radiation dose, one day later, and then once a week for four weeks. All determinations of respiration were made at a constant temperature of 20 ± 1 °C. All computations were made with log transformed data to normalize the distributions. Statistical tests are from Steel and Torrie (1960).

2.3. Small arthropod extractions

Small arthropods were extracted in a modified Macfadyen canister extractor (Macfadyen 1961), preserved in 70% alcohol, and counted.

2.4. Microbial censusing

Bacteria and fungi were censused by pour-plating ca. 40 mg. samples of soil in appropriate agar media: Thornton's standard for bacteria, and Czapek-Dox with Rose Bengal for fungi (Johnson et al. 1959).

3. Results

3.1. Soil respiration

Respiration rates are expressed as mg CO_2/day/sample, accurate to ± .004 mg. (Table 1). Definite statistical differences were found between the sample sets, both between different treatment groups, and at different times within the same group.

Mean values for respiration per core per 24 hours were compared with Duncan's multiple-range test (Table 1). The respiration rates of Table 1 are shown as curves (Figure 1) for each group over the one month time period. At the four hour reading only the laboratory 800 KR set was significantly different, showing a high initial burst of respiration (26.46 mg/24 hrs.). By the first day it had dropped to a level close to that of the set receiving 800 KR in the field, although it was still significantly higher than the other three treatments. The set receiving 2500 KR in the laboratory also had a lower respiration after the first day and was significantly different from the two groups receiving 800 KR. By the first week the 2500 KR set was significantly lower than all the others, with the 800 KR field group showing a slight rise, significantly higher than the transportation control set. The 800 KR laboratory set had, after the first week, dropped to the respiration level of the controls.

At the second week the 2500 KR set was still significantly lower in respiration rate than all the others and continued to decrease slightly as the experiment progressed. The laboratory 800 KR set also had a lower respiration rate, being significantly lower than the field 800 KR set, and by the third week was significantly lower than all other

Table 1. Multiple range-analysis of log-transformed respiration rates of irradiated and control litter-soil cores over time

Set	Date	Log mg/day	mg/day	Ranking
L 800	8— 1—68	1.4226	26.460	*
L 800	8— 2—68	1.1048	12.729	*
F 800	8— 8—68	1.0130	10.305	**
CT	8— 1—68	1.0078	10.082	***
F 800	8—15—68	0.9758	9.476	****
F 800	8— 2—68	0.9511	8.935	****
F 800	8— 1—68	0.8665	7.353	*****
F 800	8—22—68	0.8611	7.263	*****
CT	8—15—68	0.8609	7.260	*****
L 800	8— 8—68	0.8570	7.194	*****
TC	8— 1—68	0.8524	7.120	*****
L 2500	8— 1—68	0.8514	7.103	*****
F 800	8—30—68	0.8333	6.812	****
TC	8—15—68	0.8270	6.714	****
CT	8— 2—68	0.8155	6.539	****
CT	8— 8—68	0.8053	6.386	****
TC	8— 2—68	0.8016	6.333	****
TC	8— 8—68	0.7608	5.765	****
CT	8—22—68	0.7550	5.688	****
L 800	8—15—68	0.7391	5.484	***
TC	8—22—68	0.7306	5.378	***
L 2500	8— 2—68	0.6627	4.599	*
TC	8—30—68	0.6399	4.355	**
CT	8—30—68	0.6134	4.106	**
L 2500	8—15—68	0.4179	2.618	**
L 800	8—22—68	0.4091	2.565	**
L 2500	8— 8—68	0.3563	2.271	*
L 800	8—30—68	0.3316	2.139	*
L 2500	8—22—68	—0.2992	0.503	*
L 2500	8—30—68	—0.5033	0.314	*

Note: Core volume was 125 cc, area = 25 cm^2, N = 10. L 800 received 800KR in the laboratory. L 2500 received 2500 KR in the laboratory. F800 received 800 KR in the field. TC is the transporation control set. CT is the controlled temperature box set. The same set designations are used in all other tables and figures. Significance tests were made at the .05 level.

groups, except the 2500 KR, and remained this way during the fourth week. The two controls and field 800KR groups were not significantly different from each other from the second week onward.

Tests of differences in respiration rate within sample groups were compared over time (Table 2). The groups receiving 800 KR in the field and the transportation control group showed no significant changes during the four week period. The only significantly different respiration rates in the non-transport controls were the four hour and fourth week readings. The laboratory set receiving 800 KR showed a significantly high burst of respiration during the first four hours and then respiration dropped continually, the first day being significantly lower than the first four hours and the second week lower than the first day, although the first week was not. The third and fourth weeks were significantly lower than the first two weeks respiration rates.

The group receiving 2500 KR in the laboratory also showed significant drops in respiration, but it did not have the initial burst in respiration of the acute 800 group. There were three groups significantly different from one another: the four hour and one day periods, the first and second weeks, and the third and fourth weeks.

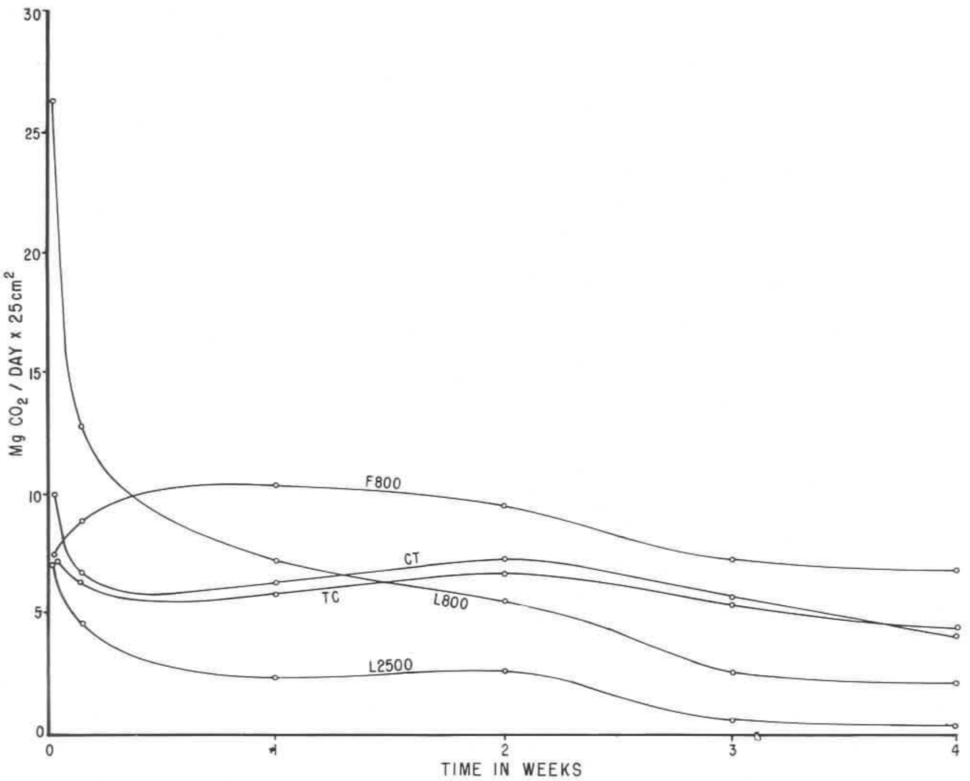

Figure 1. Respiration rates of irradiated and control cores of hardwood litter-soil, over time. Group designations are explained in Table 1.

Table 2. Multiple-range analysis of respiration rates of irradiated and control cores of litter soil over time, grouped by sets

Set	Time: 4 hours	one day	one week	second week	third week	fourth week
Chronic 800	—	—	—	—	—	—
Acute 800	—					
		—	—			
			—	—		
					—	—
Acute 2500	—	—				
			—	—		
					—	—
Transp. ctr.	—	—	—	—	—	—
Nontransp. ctr.	—	—	—	—	—	
		—	—	—	—	—

Note: Respiration rates for dates with the same line for a given set are not significantly different. Significance tests were made at the 0.05 level.

3.2. Counts of micro-organisms

At the end of week four, several cores from all five groups were extracted for microarthropods and sampled for bacteria and fungi. For all three irradiation treatments there was a virtually complete kill of small arthropods (range = 0—5 vs. a mean of ca. 150 per control core). There was a two to four-fold decrease in bacteria, with the exception of those in the 2500 KR group, which were completely killed (Table 3). In contrast, the two 800 KR groups experienced a three to ten-fold decrease in viable fungal colonies, but there was a six-fold increase in fungi in the 2500 KR set.

Table 3. Microbial populations ± 2 standard errors (S. E.) in soil cores, six weeks after irradiation

Dose (KR)	Chronic 800	Acute 800	Acute 2500	transport control	Non-transport control
Bacteria					
$\bar{x}$	26	218	0	566	986
± 2 S. E.	13	143		199	280
Fungi					
$\bar{x}$	15	4	250	45	88
± 2 S. E.	4	4	59	29	24

Note: Colonies were counted on agar pourplates (see text for details). For nos. per gram, multiply bacteria × 10^3 and fungi × 25.

4. Discussion

The 800 KR laboratory set had an initial burst of respiration and then continually decreased as time passed. The 2500 KR group lacked the initial burst, and had a more rapid decrease than the acute 800 set, coming to almost zero respiration (0.3 mg.) by the fourth week. Reasons for the differential stimulation remain unclear, although, based on previous studies, all fungi should have been killed, and not stimulated by the 2500 KR

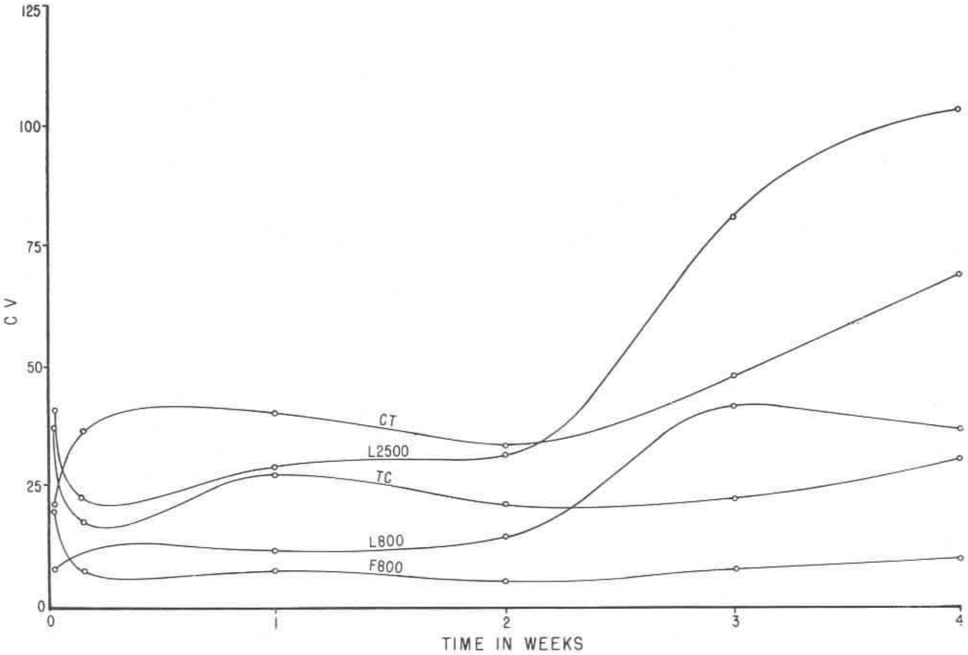

Figure 2. Coefficient of variation $\left(\frac{S}{\bar{x}}\times 100\right)$ of irradiated and control litter-soil cores. Note consistently low values for F800.

dose. In fact, a dose of ca. 800 KR was sufficient to kill all fungi in an air-dry clarion loam (JACKSON et al. 1967). Our sandy loam soil was nearly air-dry, with a moisture content of 3—4%. It is possible that the complete kill of bacteria in the 2500 KR group occurred within hours after irradiation, contributing to the marked differences in respiration in the two acute irradiation treatments. The complete bacterial kill was also found when censusing other 2500 KR litter-soil cores a few weeks later, confirming the previous finding.

The six-week (chronic) 800 KR irradiation of litter-soil by the field source had no apparent depressing effect on the rate of respiration, in contrast to the acute dosage. As a means of further comparing the acute and chronic treatments, a coefficient of variability (CV), or $(S/\bar{X} \times 100)$ was calculated for all the sample sets (Figure 2). The field 800 KR set had a persistently lower CV, compared to the other sets. This may be due to two factors: (1) a radiation-induced migration of certain organisms away from the higher

Table 4. Analysis of variance of log respiration rates of irradiated and control sets of soil samples using the F test

Time after Irradiation	Set	S^2	Ranking
Four hours			
	L 2500	0.12134	*
	TC	0.10443	*
	CT	0.04254	**
	F 800	0.03544	**
	L 800	0.01412	*
One day			
	CT	0.09160	*
	TC	0.03450	**
	L 2500	0.01381	**
	L 800	0.01198	**
	F 800	0.00486	*
One week			
	CT	0.10462	*
	TC	0.04382	*
	L 800	0.01076	*
	L 2500	0.01072	*
	F 800	0.00600	*
Two weeks			
	CT	0.08426	*
	TC	0.03031	**
	L 2500	0.01727	*
	L 800	0.01171	*
	F 800	0.00303	*
Three weeks			
	CT	0.13411	*
	L 2500	0.05963	**
	L 800	0.02986	*
	TC	0.02787	*
	F 800	0.00460	*
Four weeks			
	L 2500	0.27791	*
	CT	0.18221	*
	TC	0.04021	*
	L 800	0.01529	**
	F 800	0.00816	*

Note: Ranking is by the multiple-range test at the .05 level. Set abbreviations are same as in Table 1.

radiation area; or (2) the killing of low tolerance microflora, leaving a more homogeneous and simpler population. Under this hypothesis, the organisms remaining might make use of the increased resources available to them increasing their respiration slightly, as a result of lessened competition.

The laboratory 800 KR set also had a low CV at first, but it went up after the third week. The high CV of the third and fourth weeks readings of the 2500 KR cores is similar to that of the acute 800 KR group and is most probably due to respiration being so low relative to the sample standard deviations.

In order to test statistically the variability between treatments, an analysis of variance using a ratio of variances was done for each of the sets (Table 4). This showed that the 800 KR field group had a significantly lower variance than the controls or acutely-irradiated cores.

5. Conclusions

The study shows that although the long term (chronic) irradiation does not affect the rate of respiration in the same fashion as the short period (acute) laboratory doses, it reduces the variability, and initially increases the rates of CO_2 evolution in a significant manner. Further analyses of changes in species or entire microbial groups should prove useful in secondary production studies of litter-soil when compared with changes of respiration rate.

6. Acknowledgments

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7. Summary

Groups of cores of hardwood litter-soil were treated either in the field with low-dosage gamma radiation (chronic) or in the laboratory, with a more intense gamma source (acute). The irradiated cores and non-irradiated controls were sealed in polyethylene, and carbon dioxide evolution followed over a one month period.

The long-term, chronic dosage was sligthly stimulatory but less variable after a one week delay, whereas the acute treatments were depressed after an initial burst in the 800 Kilo-Roentgen (KR) set. Bacterial numbers in litter-soil receiving a chronic 800 KR dose were one-tenth those in the acute treatment, but were completely eliminated by an acute dosage of 2500 KR. Stressing litter-soil with gamma radiation offers promise for secondary productivity studies in systems with reduced species complexity.

8. Literature

Bowen, H. J. M., and P. A. Cawse, 1964. Effects of ionizing radiation on soils and subsequent crop growth. Soil Sci. **97**, 252—259.

Cawse, P. A., 1967. Effects of low sub-sterilising doses of gamma radiation on carbon, nitrogen and phosphorus in fresh soils. J. Sci. Fd. Agric. **18**, 388—391.

Coleman, D. C., and A. Macfadyen, 1966. The recolonization of gamma-irradiated soil by small arthropods. Oikos **17**, 62—70.

Cowley, G. T., 1969. Effect of irradiation on the microfungal populations of six litter species in the Luquillo Experimental Forest. In: Odum, H. T. (Ed.): A Tropical Rain Forest. In press.

Edwards, C. A., 1968. The effects of gamma irradiation on populations of soil invertebrates. Proc. Second Intl. Cong. Radioecology, Ann. Arbor. In press.

Eno, C. F., and H. Popenoe, 1963. The effect of gamma radiation on the availability of nitrogen and phosphorus in soil. Soil Sci. Soc. Amer. Proc. **27** (3), 299—301.

— 1964. Gamma radiation compared with steam and methyl bromide as a soil sterilizing agent. Soil Sci. Soc. Amer. Proc. **28** (4), 533—535.

Jackson, N. E., J. C. Corey, L. R. Frederick and J. C. Picken, Jr., 1967. Gamma irradiation and the microbial populations of soils at two water contents. Soil Sci. Soc. Amer. Proc. **31** (4), 491—494.

Johnson, L. F., E. A. Curl, J. H. Bond and H. A. Fribourg, 1959. Methods for studying soil microflora-plant disease relationships. Burgess Pub. Co., Minneapolis.

Macfadyen, A., 1961. Improved funnel-type extractors for soil arthropods. J. Anim. Ecol. **30**, 171—184.

McCormick, J. F., and F. B. Golley, 1966. Irradiation of natural vegetation. An experimental facility, procedures and dosimetry. Health Physics **12**, 1467—1474.

Monk, C. D., 1966. Effects of short-term gamma irradiation on an old field. Radiation Bot. **6**, 329—335.

Popenoe, H., and C. F. Eno, 1962. The effect of gamma radiation on the microbial population of the soil. Soil. Soc. Amer. Proc. **26** (2), 164—167.

Steel, R. G., and J. H. Torrie, 1960. Principles and Procedures of Statistics. McGray-Hill Book Company, Inc., New York, Pages 20, 82—83, and 101—109.

Witkamp, M., 1961. Influence of radiation from an unshielded reactor on a natural microflora. ORNL-TM-29: 1—8. (Technical publication of Oak Ridge National Labs.).

— 1963. Microbial population of leaf litter in relation to environmental conditions and decomposition. Ecology **44**, 370—377.

Woodwell, G. M., and A. L. Rebuck, 1967. Effects of chronic gamma radiation on the structure and diversity of an oak-pine forest. Ecol. Monogr. **37**, 53—69.

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